

## DROUGHT RESPONSE OF TWO MEXICAN OAK SPECIES, *QUERCUS LACEYI* AND *Q. SIDEROXYLA* (FAGACEAE), IN RELATION TO ELEVATIONAL POSITION<sup>1</sup>

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To investigate the relationship between the altitudinal distribution of *Quercus laceyi* and *Q. sideroxyla* and their physiological responses to drought, we measured relative water content (RWC), water potentials ( $\Psi_{\text{predawn}}$  and  $\Psi_{\text{midday}}$ ), photosynthesis ( $A_{\text{max}}$ ), stomatal conductance ( $g$ ), chlorophyll fluorescence ( $F_v/F_m$ ), and spectral reflectance (400–1100 nm) five times during a 7 wk acute drought. *Quercus laceyi* was drought tolerant, while *Q. sideroxyla* was a drought avoider; *Q. laceyi* tolerated lower RWC (*Q. sideroxyla* = 54%, *Q. laceyi* = 44%),  $\Psi_{\text{pd}}$  (*Q. sideroxyla* = –2.6 MPa, *Q. laceyi* = –3.3 MPa), and  $\Psi_{\text{md}}$  (*Q. sideroxyla* = –4.5 MPa, *Q. laceyi* = –6.6 MPa). The  $F_v/F_m$  also declined first in *Q. sideroxyla* in wk 6, whereas  $F_v/F_m$  did not decline in *Q. laceyi* until wk 7.  $A_{\text{max}}$  and  $g$  fell in wk 4, 6, and 7 in drought seedlings of both species, suggesting a decline in CO<sub>2</sub> assimilation during the drought. Leaf spectral reflectance increased with time in response to decreases in leaf photosynthetic pigment concentrations in latter weeks of the drought. The results suggest a close association between the altitudinal distributions of these species and their adaptation to water stress.

**Key words:** chlorophyll fluorescence; elevation gradient; Fagaceae; photosynthetic efficiency; *Quercus*; spectral reflectance; stress physiology; water relations.

The genus *Quercus* (oaks; Fagaceae) is one of the most diverse groups of temperate trees, with greater than 500 species in the temperate and subtropical latitudes of the Old and New worlds (Nixon, 1993). Mexico represents the origin of oak diversification in North America and contains the highest oak diversity in the world (Muller and McVaugh, 1972; Rzedowski, 1978; González, 1993; Nixon, 1993). Oak and pine–oak forests cover almost half (45%) of the temperate coniferous forest of Mexico (World Forest Institute, 1994) and are globally significant centers of vascular plant diversity and of endemism in both plant and animal species (Manuel-Toledo and Jesús-Ordóñez, 1993). However, the mechanisms underlying oak diversification across Mexico are poorly understood.

With 63% of its surface area covered by desert, Mexico contains the most arid regions in North America. In these extreme environments, water is the predominant factor limiting carbon gain and primary production (Noy-Mier, 1973; DeLucia and Schlesinger, 1991; Lajtha and Getz, 1993; Kozlowski and Pallardy, 1997). Yet, no published information quantifies the variability of oak drought tolerance in relation to species distribution patterns in Mexico.

Oaks are some of the most drought tolerant trees of the temperate latitudes (Abrams, 1990; Ogaya and Peñuelas, 2003; Aranda et al., 2005). North American oaks are adapted to drought prone sites through a variety of mechanisms including drought avoidance and drought tolerance (Abrams, 1990, 1996), and the distribution of oak species in the United States is tightly linked to the physiological, anatomical, and morpho-

logical variation among species and across sites (Kubiske and Abrams, 1992; Ashton and Berlyn, 1994).

Understanding the relationship between oak physiology and moisture regimes is especially important in arid regions of North America, where precipitation is already greatly exceeded by evaporative demand and where the water deficit might be increased as a result of global warming (Houghton et al., 2001; Loik et al., 2004). Some reports describe the response of several oak species in the Rocky Mountains (Neilson and Wullstein, 1987; Dickson and Tomlinson, 1996; Ehleringer and Phillips, 1996; Balok and St. Hilaire, 2002), yet to our knowledge, no prior research explains the effects of moisture regime variability across mountain ranges on oak ecophysiology and adaptations to water stress in the oak species complexes in Mexico.

The objective of this study was to measure the physiological responses of a high and a low elevation endemic Mexican oak species of the genus *Quercus* to an experimental acute drought similar to that experienced by seedlings in the field before the onset of the North American Monsoon System (NAMS), which occurs in the spring across the southwestern United States and Northern Mexico (Adams and Comrie, 1997). Our intent was to investigate the physiological mechanisms responsible for the distribution of two oak species, *Quercus laceyi* Small (subgenus *Leucobalanus*; Fagaceae) and *Q. sideroxyla* Humb. et Bonpl (subgenus *Erythrobalanus*; Fagaceae), on different sites within the same mountain range.

Seedling performance was examined under two moisture regimes (unlimited water and drought) on two species inhabiting sites that vary dramatically in local environmental conditions. *Quercus laceyi* is the lowest elevation tree of any species in the Sierra del Carmen, Coahuila, Mexico, and it is the dominant low-elevation oak in savannahs and riparian areas. It is a medium stature tree that ranges from 3–8 m in height, and it typically colonizes hot, dry sites. *Quercus sideroxyla* is the dominant high-elevation oak in the Sierra del Carmen. This species colonizes cooler, wetter sites compared to *Q. laceyi*. It coexists with a mixture of other oak and conifer

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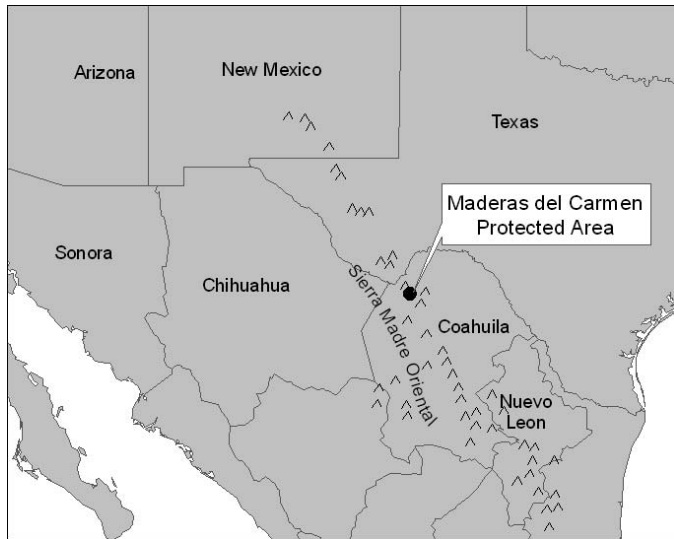


Fig. 1. Location of the Maderas del Carmen study area in Coahuila, Mexico.

species in closed canopy forests that reach over 30 m in height. The two species have overlapping elevational ranges, but occur in different forest types giving them spatially segregated distributions.

By measuring a suite of physiological parameters during an imposed 7-wk drought, we tested the hypothesis that these species have distinct physiological responses to drought that reflect their different altitudinal distributions. Differences in the physiological response of these species to drought may explain the niche differentiation or specialization of oaks on different sites within the same mountain range (May and MacArthur, 1972; Pianka, 1976). Furthermore, this research has the potential to elucidate the mechanisms responsible for the high oak diversity in northern Mexico.

## MATERIALS AND METHODS

**Experimental conditions and experimental design**—Ripe acorns were collected from 20 mature trees of each species in the Sierra del Carmen of Coahuila, Mexico in November 2004 (Fig. 1). In montane woodlands of this region, acorns mature in the fall, germinate, and establish under frequent winter precipitation, and are then exposed to a prolonged drought from March until mid-July when precipitation returns with the NAMS. While stratification is necessary for the germination of some oaks of the subsection *Erythrobalanus*, oaks in the southwestern United States and Mexico germinate readily after maturation (Nyandiga and McPherson, 1992).

Six hundred acorns were submerged in water and placed in a 5°C refrigerator for 24 h to promote germination. Acorns were planted in a Metromix seedbed (Scott's Co., Marysville, Ohio, USA) inside a greenhouse with a daily temperature range of 18–42°C and mean relative humidity of 20% (12–45% range) in New Haven, Connecticut on 22 March 2005 (lat. 41°16' N; long. 72°55' W). Air temperature and relative humidity were measured with a Hobo Micro Station Data Logger (Onset Computer, Bourne, Massachusetts, USA). Photosynthetically active radiation (PAR) was measured at 30-min intervals across the greenhouse bench used in the experiment with a HOBO Photosynthetic Light (PAR) Smart Sensor (Onset Computer) attached to the data logger. The mid-day peak in PAR varied from 1100 to 1561  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

Eighty percent of the acorns planted in our study germinated under greenhouse conditions. No information exists that describes the germination

success of these two species in the field. However, two similar species, *Q. arizonica* Sarg. (subgenus *Leucobalanus*) and *Q. emoryi* Torr. (subgenus *Erythrobalanus*), also in the Sierra del Carmen, had 72% and 21% germination, respectively, under field conditions in Arizona (Nyandiga and McPherson, 1992).

After 3 wk, when the radical was 3 cm long, germinants were transplanted to 10-cm plastic pots. Seedlings were transplanted again to 18-cm plastic pots filled with Metromix growing medium on 18 September 2005. A modified Hoagland's nutrient solution (Hoagland and Arnon, 1950) was applied monthly for 3 mo to all plants before the onset of the experiment. Metromix growing medium was chosen because it is a commercially available medium that drains well. The soils of the natural habitats of the two species are generally shallow to moderately deep and are volcanic in origin. The underlying rocks are predominantly extrusives, consisting of lavas and pyroclastics.

On 21 March 2006, 120 healthy seedlings of each species (240 total) were divided into two treatments, drought and control, using a completely randomized sampling design. The date of experiment onset was chosen to simulate the drought experienced by these species in the field before the onset of the NAMS (Adams and Comrie, 1997). High temperatures and little precipitation characterize the natural drought. No climatic data exist for the Sierra del Carmen, but mean annual precipitation for the Chisos Basin approximately 150 km to the north is 70 cm (range 32–135 cm from 1948–2003). Mean January precipitation is 1.5 cm (range 0–2.5 cm), mean precipitation in March before the NAMS is 0.1 cm (range 0–3.7 cm), and mean precipitation in July during the NAMS is 8.0 cm (range 0.2–20.5 cm). Mean monthly minimum temperatures are 1.8°C in January, 6.3°C in March and 17.0°C in July. Maximum temperatures are 14.1°C in January, 20.9°C in March, and 29.1°C in July. Relative humidity during the drought (March–July) fluctuates between 10 and 25%. Daily greenhouse temperatures and relative humidity were not controlled during the experiment, although the mean relative humidity and temperatures were within the ranges experienced by seedlings in the field during the natural drought.

The experimental unit was a single seedling in a pot, and soil moisture was monitored using gravimetric methods. All pots were watered to saturation (field capacity) at the onset of the experiment and were weighed. Control seedling pots were watered to 100% of field capacity three times per wk by bringing them to their field capacity weight. After initial watering, seedlings in the drought treatment were exposed to an acute drought, where water was completely withheld for the duration of the experiment. Drought seedlings were weighed weekly to determine their percentage of field capacity mass. Drought seedlings reached 10% field capacity at the end of the experiment (reached 48 d after the onset of the drought treatment).

**Water relations**—Predawn (0400 to 0500 hours) and midday (1200 to 1300 hours) water potentials ( $\Psi_{\text{pd}}$  and  $\Psi_{\text{md}}$ ) (MPa) were measured at the onset of the experiment, and at 2, 4, 6, and 7 wk into the experiment. Three individuals of each species-treatment combination ( $N = 12$ ) were destructively sampled at predawn for the determination of  $\Psi_{\text{pd}}$  using a pressure chamber (PMS Instruments, Corvallis, Oregon, USA). Water potential measurements were repeated at 1200 hours on the same days on 12 additional individuals to obtain midday  $\Psi_{\text{md}}$  values.

Leaf relative water content (RWC) was determined for five leaf discs from leaves on the same seedlings used in the  $\Psi$  measurements. Leaf discs were cut and immediately weighed on a digital balance (A & D Systems, Milpitas, California, USA) to obtain their fresh mass (FM). Leaf discs were then saturated to full turgor by immersing them in distilled water in the dark for 24 h. Leaves were reweighed to obtain their turgid mass (TM) and put into an oven at 70°C for 3 d to obtain their dry mass (DM). RWC was calculated as:  $\text{RWC} = (\text{FM} - \text{DM})/(\text{TM} - \text{DM}) \times 100$ .

**Repeated physiological measurements**—Carbon assimilation ( $A$ ), stomatal conductance ( $g$ ), intracellular  $\text{CO}_2$  concentrations ( $C_i$ ), chlorophyll fluorescence ( $F_v/F_m$ ), and spectral reflectance were measured on a subset of 40 individuals (10 individuals from each species-treatment combination) at each of the sampling intervals (onset, 2 wk, 4 wk, 6 wk, and 7 wk) during the drought. A mature, fully expanded leaf was chosen at the onset of the experiment for the repeated physiological measurements, and the same leaf was used at each of the subsequent sampling intervals. All repeated physiological measurements were conducted between 0800 and 1130 hours.

Maximum photosynthesis ( $A_{\text{max}}$ ),  $g$ , and  $C_i$  were measured in situ using an open-system portable infrared gas analyzer (Welles, 1986) (LI-6400, Li-Cor, Lincoln, Nebraska, USA). Measurements of  $A_{\text{max}}$  were taken under saturating

TABLE 1.  $P$  values for effects included in the analysis of variance (ANOVA) for the various physiological measurements of *Quercus sideroxylla* and *Q. laceyi* using mixed models for (A) repeated physiological traits and  $P$  values for one-way ANOVAs for (B) destructively sampled physiological traits.

| (A) Repeated measures physiological traits     |             |             |        |            |           |                      |        |            |            |        |           |
|------------------------------------------------|-------------|-------------|--------|------------|-----------|----------------------|--------|------------|------------|--------|-----------|
| Fixed factor                                   | SIPI        | WBI         | chlNDI | NDVI       | REP       | Root collar diameter | Height | No. leaves | $A_{\max}$ | $g$    | $F_v/F_m$ |
| Treatment                                      | 0.0529      | 0.0008      | 0.0513 | 0.0001     | 0.0001    | 0.0017               | 0.0100 | 0.3632     | 0.0000     | 0.0000 | 0.0000    |
| Species                                        | 0.0141      | 0.8663      | 0.0002 | 0.0023     | 0.0040    | 0.2259               | 0.0260 | 0.0036     | 0.0000     | 0.0000 | 0.0012    |
| Time                                           | 0.0778      | 0.0001      | 0.0001 | 0.0001     | 0.0001    | 0.8178               | 0.8000 | 0.1058     | 0.0000     | 0.0000 | 0.0000    |
| (B) Destructively sampled physiological traits |             |             |        |            |           |                      |        |            |            |        |           |
| Fixed factor                                   | $\Psi_{pd}$ | $\Psi_{md}$ | RWC    | Shoot mass | Root mass | R : S                |        |            |            |        |           |
| Treatment                                      | 0.0001      | 0.0001      | 0.0120 | 0.0030     | 0.0001    | 0.8625               |        |            |            |        |           |
| Species                                        | 0.6489      | 0.0057      | 0.0010 | 0.5797     | 0.1263    | 0.3723               |        |            |            |        |           |
| Time                                           | 0.0001      | 0.0001      | 0.0001 | n/a        | n/a       | n/a                  |        |            |            |        |           |
| Treatment $\times$ time                        | 0.0001      | 0.0200      | 0.0120 | n/a        | n/a       | n/a                  |        |            |            |        |           |
| Treatment $\times$ species                     | 0.5385      | 0.5734      | 0.2000 | 0.8748     | 0.0843    | 0.4326               |        |            |            |        |           |
| Species $\times$ time                          | 0.4726      | 0.0457      | 0.2530 | n/a        | n/a       | n/a                  |        |            |            |        |           |
| Species $\times$ time $\times$ treatment       | 0.9220      | 0.8220      | 0.1209 | n/a        | n/a       | n/a                  |        |            |            |        |           |

Note:  $A_{\max}$  = maximum photosynthesis;  $g$  = stomatal conductance;  $F_v/F_m$  = chlorophyll fluorescence to maximal chlorophyll fluorescence; chlNDI = chlorophyll normalized difference vegetation index; NDVI = normalized difference vegetation index; REP = red edge position; RWC = relative water content; SIPI = structure independent pigment index; WBI = water band index;  $\Psi_{pd}$  = pre-dawn water potential;  $\Psi_{md}$  = mid-day water potential.

light ( $1800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) using the LED attachment for the LI-6400 photosynthesis system. Light curves were used to verify saturating light levels for the two species (data not shown).

The maximum efficiency of photosystem II (PSII) chemistry ( $F_v/F_m$ ) was measured in situ on attached leaves using a portable pulse-modulated fluorometer (OS 500, Opti-Sciences, Hudson, New Hampshire, USA). Leaves were dark-adapted for 1 h, which was sufficient to allow complete relaxation of energy-dependent quenching. The ratio  $F_v/F_m$  was calculated as  $(F_m - F_o)/F_m$ , where  $F_m$  and  $F_o$  are the maximum and basal fluorescence yields, respectively, of dark-adapted leaves (Gentry et al., 1989).

Spectral reflectance of the upper leaf surface (adaxial side) was measured using a UNISpec Spectral Analysis System (PP Systems, Haverhill, Massachusetts, USA) over the range of 400–1000 nm with a 2.0 mm diameter foreoptic and an internal 6.8 W halogen lamp. Standard controls were dark and a spectralon reflectance white standard. The UNISpec unit was recalibrated after every 10 measurements using the reflectance white standard. Leaves were held in a black polyvinyl chloride (PVC) clip at a  $60^\circ$  angle relative to the foreoptic for scanning ( $R_\lambda$  = leaf radiance at wavelength  $\lambda$ ). We used four indices based on the reflectance properties of plant pigments and their biochemical components to identify differences in the reflectance spectra according to species and treatment: (1) a measure of carotenoids: chlorophyll  $a$  ratio, the structure independent pigment index (Peñuelas et al., 1995) ( $\text{SIPI} = (R_{800} - R_{445})/(R_{800} - R_{680})$ ); (2) the normalized difference vegetation index,  $\text{NDVI} = (R_{750} - R_{680})/(R_{750} + R_{680})$ ; (3) a revised version of NDVI that is sensitive to chlorophyll  $a$  concentrations (Gitelson and Merzlyak, 1996),  $\text{chlNDI} = (R_{750} - R_{705})/(R_{750} + R_{705})$ ; and (4) a water band index,  $\text{WBI} = (R_{900} + (R_{900} - R_{830}))/R_{970}$ , a measure of vegetation water content (Riedell and Blackmer, 1999).

The red edge position (REP) is the name given to the change in reflectance in the 670–780 nm range of vegetation spectra caused by strong chlorophyll absorption by photosystems I and II and by internal leaf scattering. It is defined as the wavelength of the inflection point of the reflectance slope across this region and is indicative of leaf chlorophyll content. Under stress, the red edge position shifts toward the shorter wavelengths as a result of senescence or stress-induced chlorosis (Rock et al., 1988; Peñuelas et al., 1994; Thorhaug et al., 2006). REP was calculated using the four-points formula developed by Guyot et al. (1992), which performs well in comparison to other more computationally intensive REP measures (Pu et al., 2003). The REP was determined by calculating the reflectance at the red edge inflection point ( $R_{re}$ ) given by  $R_{re} = (R_{670} + R_{780})/2$ , and then by deriving the red edge wavelength ( $\lambda_{re}$ ) given by  $\lambda_{re} = 700 + 40 \cdot [(R_{re} - R_{700})/(R_{740} - R_{700})]$  where  $R_{670}$ ,  $R_{700}$ ,  $R_{740}$ , and  $R_{780}$  are the reflectance values at 670, 700, 740, and 780 nm wavelength, respectively.

**Growth and biomass allocation**—Seedling growth and dry mass gain were determined at the end of the experiment for 20 individuals in each species-treatment combination ( $N = 80$ ) in a stratified random manner. Species height and leaf number were determined on the same individuals at the onset of the experiment and at wk 4 and 7. Root collar diameter was measured at wk 4 and 7. Seedlings were harvested at the end of the experiment, and leaves, stems, and roots were separated, dried at  $70^\circ\text{C}$  for 3 d, and then dried at  $90^\circ\text{C}$  until a constant mass was reached. Whole shoot and root dry masses were recorded for each individual, and the root : shoot ratio (R : S) was calculated. The differences between root and shoot masses in the control and the drought treatment were also determined.

**Statistical analyses**—Multifactor ANOVAs were used to determine the effects of species, treatment, and time on seedling performance. All statistical analyses were performed using the R Statistical Language (R Development Core Team, 2005). RWC,  $\Psi_{pd}$ ,  $\Psi_{md}$ , dry plant matter mass (leaves, stems, roots), and root : shoot measurements were analyzed using a general linear model (GLM) procedure with fixed effects of species, treatment, and time and the interaction effects of species  $\times$  treatment, species  $\times$  time, treatment  $\times$  time, and species  $\times$  treatment  $\times$  time. Differences between species-treatment combinations at each sampling interval were determined using a Tukey's test of significant difference. Differences between  $\Psi_{pd}$  and  $\Psi_{md}$  measurements were also assessed using a GLM. Repeated measurements of  $A_{\max}$ ,  $C_i$ ,  $g$ , SIPI, PRI, chlNDI, NDVI, WBI, RE,  $F_v/F_m$ , and seedling growth (number of leaves, height, root collar diameter) were analyzed using separate ANOVAs with repeated measures by a mixed models procedure with species and treatment as fixed effects and time as a random effect. Mixed models were used rather than repeated measures GLMs to account for differences in the covariance structure of the repeated measures. A regression was also performed to assess the relationship between  $A_{\max}$  and  $g$ .

## RESULTS

**Water relations**—The ANOVAs (Table 1) revealed significant ( $P < 0.0001$ ) effects of treatment, time, and the interaction of treatment  $\times$  time for  $\Psi_{md}$ , which demonstrated the impact of the drought treatment on the seedlings of both species, and showed the accelerating impact of drought over time. Species-level differences for  $\Psi_{md}$  were significant at

TABLE 2. Means by species and treatment for different (A) physiological measurements; (B) reflectance indices; and (C) growth measurements of *Quercus sideroxyla* (QUSI) and *Q. laceyi* (QULA). Means ( $\pm$ SE) are presented in relation to Table 1. Lowercase letters beside the SEs represent significant differences between species and treatments at the 5% level according to Tukey's comparisons.

| (A) Physiological measurement                                     |      |         | Species | Treatment | Week 0               | Week 2             | Week 4             | Week 6              | Week 7              |
|-------------------------------------------------------------------|------|---------|---------|-----------|----------------------|--------------------|--------------------|---------------------|---------------------|
| RWC (%)                                                           | QULA | Control |         |           | 67.0 $\pm$ 1.9 a     | 72.2 $\pm$ 2.15 a  | 67.1 $\pm$ 1.16 a  | 55.8 $\pm$ 0.69 a   | 59.2 $\pm$ 0.76 a   |
|                                                                   |      | QUSI    | Control |           | 75.3 $\pm$ 1.4 b     | 72.5 $\pm$ 0.97 a  | 76.5 $\pm$ 1.63 b  | 66.5 $\pm$ 1.10 a   | 63.9 $\pm$ 0.13 a   |
|                                                                   | QULA | Drought |         |           | 61.8 $\pm$ 1.7 a     | 58.3 $\pm$ 1.87 a  | 57.5 $\pm$ 1.22 a  | 44.0 $\pm$ 0.96 a   | 44.0 $\pm$ 2.78 a   |
|                                                                   |      | QUSI    | Drought |           | 81.2 $\pm$ 1.0 b     | 74.8 $\pm$ 1.50 a  | 72.4 $\pm$ 0.52 b  | 53.3 $\pm$ 2.53 a   | 54.5 $\pm$ 1.61 a   |
|                                                                   | QULA | Control |         |           | -0.3125 $\pm$ 0.01 a | -0.37 $\pm$ 0.01 a | -0.28 $\pm$ 0.02 a | -0.2 $\pm$ 0.00 a   | -1.31 $\pm$ 0.21 ac |
|                                                                   |      | QUSI    | Control |           | -0.225 $\pm$ 0.01 b  | -0.32 $\pm$ 0.00 a | -0.60 $\pm$ 0.00 a | -0.225 $\pm$ 0.00 a | -0.35 $\pm$ 0.00 ac |
| $\Psi_{pd}$ (MPa)                                                 | QULA | Drought |         |           | -0.35 $\pm$ 0.03 a   | -0.46 $\pm$ 0.02 a | -0.60 $\pm$ 0.00 b | -2.85 $\pm$ 0.25 b  | -3.35 $\pm$ 0.35 ac |
|                                                                   | QUSI | Drought |         |           | -0.19 $\pm$ 0.00 b   | -0.50 $\pm$ 0.02 a | -0.51 $\pm$ 0.03 b | -1.86 $\pm$ 0.29 b  | -2.6 $\pm$ 0.09 b   |
|                                                                   | QULA | Control |         |           | -0.516 $\pm$ 0.13 a  | -0.88 $\pm$ 0.38 a | -0.70 $\pm$ 0.00 a | -0.6 $\pm$ 0.17 a   | -1.7 $\pm$ 1.99 ac  |
|                                                                   | QUSI | Control |         |           | 0.25 $\pm$ 0.07 b    | -0.82 $\pm$ 0.34 a | -0.47 $\pm$ 0.23 a | -0.6 $\pm$ 0.20 a   | -0.6 $\pm$ 0.00 a   |
| $\Psi_{md}$ (MPa)                                                 | QULA | Drought |         |           | -0.567 $\pm$ 0.10 a  | -0.83 $\pm$ 0.15 a | -1.13 $\pm$ 0.56 a | -2.2 $\pm$ 0.95 b   | -6.6 $\pm$ 1.56 bc  |
|                                                                   | QUSI | Drought |         |           | -0.542 $\pm$ 0.04 b  | -0.86 $\pm$ 0.05 a | -1.00 $\pm$ 0.95 a | -1.4 $\pm$ 1.95 b   | -4.5 $\pm$ 0.76 b   |
|                                                                   | QULA | Control |         |           | 10.374 $\pm$ 0.55 a  | 10.49 $\pm$ 0.51 a | 8.18 $\pm$ 0.48 a  | 9.532 $\pm$ 0.66 a  | 7.923 $\pm$ 0.71 a  |
|                                                                   | QUSI | Control |         |           | 15.610 $\pm$ 0.48 b  | 14.29 $\pm$ 0.57 b | 10.57 $\pm$ 0.70 a | 13.442 $\pm$ 0.63 a | 11.817 $\pm$ 0.56 a |
| $A_{max}$ ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) | QULA | Drought |         |           | 4.083 $\pm$ 0.33 a   | 4.96 $\pm$ 0.31 a  | 4.20 $\pm$ 0.66 b  | 1.194 $\pm$ 0.19 b  | 0.409 $\pm$ 0.06 b  |
|                                                                   | QUSI | Drought |         |           | 14.324 $\pm$ 0.76 b  | 12.39 $\pm$ 0.53 b | 8.59 $\pm$ 0.60 b  | 1.847 $\pm$ 0.47 b  | 0.758 $\pm$ 0.33 b  |
|                                                                   | QULA | Control |         |           | 0.187 $\pm$ 0.01 a   | 0.17 $\pm$ 0.01 a  | 0.09 $\pm$ 0.01 a  | 0.144 $\pm$ 0.01 a  | 0.107 $\pm$ 0.01 a  |
|                                                                   | QUSI | Control |         |           | 0.286 $\pm$ 0.01 b   | 0.21 $\pm$ 0.01 b  | 0.14 $\pm$ 0.01 a  | 0.211 $\pm$ 0.01 a  | 0.180 $\pm$ 0.01 a  |
| $g$ ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )       | QULA | Drought |         |           | 0.244 $\pm$ 0.01 a   | 0.12 $\pm$ 0.01 a  | 0.05 $\pm$ 0.01 b  | 0.011 $\pm$ 0.00 b  | 0.002 $\pm$ 0.00 b  |
|                                                                   | QUSI | Drought |         |           | 0.255 $\pm$ 0.02 b   | 0.20 $\pm$ 0.01 b  | 0.09 $\pm$ 0.01 b  | 0.008 $\pm$ 0.01 b  | 0.007 $\pm$ 0.00 b  |
|                                                                   | QULA | Control |         |           | 0.838 $\pm$ 0.00 a   | 0.83 $\pm$ 0.00 a  | 0.82 $\pm$ 0.00 a  | 0.822 $\pm$ 0.00 a  | 0.782 $\pm$ 0.02 a  |
|                                                                   | QUSI | Control |         |           | 0.850 $\pm$ 0.00 a   | 0.85 $\pm$ 0.00 b  | 0.83 $\pm$ 0.01 a  | 0.810 $\pm$ 0.01 b  | 0.850 $\pm$ 0.00 a  |
| $F_v/F_m$                                                         | QULA | Drought |         |           | 0.842 $\pm$ 0.00 a   | 0.81 $\pm$ 0.01 a  | 0.80 $\pm$ 0.01 a  | 0.783 $\pm$ 0.01 ac | 0.276 $\pm$ 0.05 b  |
|                                                                   | QUSI | Drought |         |           | 0.832 $\pm$ 0.01 a   | 0.84 $\pm$ 0.00 b  | 0.81 $\pm$ 0.01 a  | 0.525 $\pm$ 0.06 bc | 0.265 $\pm$ 0.06 b  |
| (B) Reflectance index                                             |      |         | Species | Treatment | Week 0               | Week 2             | Week 4             | Week 6              | Week 7              |
| NDVI                                                              | QULA | Control |         |           |                      | 0.776 $\pm$ 0.00 a | 0.799 $\pm$ 0.00 a | 0.799 $\pm$ 0.00 a  | 0.743 $\pm$ 0.02 a  |
|                                                                   | QUSI | Control |         |           |                      | 0.827 $\pm$ 0.01 b | 0.809 $\pm$ 0.02 a | 0.809 $\pm$ 0.02 a  | 0.721 $\pm$ 0.04 a  |
|                                                                   | QULA | Drought |         |           |                      | 0.780 $\pm$ 0.00 a | 0.792 $\pm$ 0.00 a | 0.792 $\pm$ 0.00 a  | 0.685 $\pm$ 0.02 a  |
|                                                                   | QUSI | Drought |         |           |                      | 0.828 $\pm$ 0.00 b | 0.845 $\pm$ 0.00 a | 0.845 $\pm$ 0.00 a  | 0.593 $\pm$ 0.05 a  |
| chlNDI                                                            | QULA | Control |         |           |                      | 0.452 $\pm$ 0.00 a | 0.438 $\pm$ 0.01 a | 0.438 $\pm$ 0.01 a  | 0.424 $\pm$ 0.01 a  |
|                                                                   | QUSI | Control |         |           |                      | 0.543 $\pm$ 0.01 b | 0.527 $\pm$ 0.02 b | 0.527 $\pm$ 0.02 b  | 0.536 $\pm$ 0.00 a  |
|                                                                   | QULA | Drought |         |           |                      | 0.445 $\pm$ 0.00 a | 0.454 $\pm$ 0.00 a | 0.454 $\pm$ 0.00 a  | 0.310 $\pm$ 0.02 b  |
|                                                                   | QUSI | Drought |         |           |                      | 0.500 $\pm$ 0.01 b | 0.509 $\pm$ 0.01 b | 0.509 $\pm$ 0.01 b  | 0.260 $\pm$ 0.03 b  |
| WBI                                                               | QULA | Control |         |           |                      | 1.029 $\pm$ 0.00 a | 1.028 $\pm$ 0.00 a | 1.028 $\pm$ 0.00 a  | 1.019 $\pm$ 0.00 a  |
|                                                                   | QUSI | Control |         |           |                      | 1.030 $\pm$ 0.00 b | 1.028 $\pm$ 0.00 a | 1.028 $\pm$ 0.00 a  | 1.022 $\pm$ 0.00 a  |
|                                                                   | QULA | Drought |         |           |                      | 1.028 $\pm$ 0.00 a | 1.027 $\pm$ 0.00 a | 1.027 $\pm$ 0.00 a  | 1.005 $\pm$ 0.00 b  |
|                                                                   | QUSI | Drought |         |           |                      | 1.030 $\pm$ 0.00 b | 1.030 $\pm$ 0.00 a | 1.030 $\pm$ 0.00 a  | 0.996 $\pm$ 0.00 b  |
| SIPI                                                              | QULA | Control |         |           |                      | 1.033 $\pm$ 0.00 a | 1.028 $\pm$ 0.00 a | 1.028 $\pm$ 0.00 a  | 0.994 $\pm$ 0.00 ac |
|                                                                   | QUSI | Control |         |           |                      | 1.035 $\pm$ 0.00 b | 1.048 $\pm$ 0.01 a | 1.048 $\pm$ 0.01 a  | 1.016 $\pm$ 0.00 ac |
|                                                                   | QULA | Drought |         |           |                      | 1.030 $\pm$ 0.00 a | 1.025 $\pm$ 0.00 a | 1.025 $\pm$ 0.00 a  | 1.005 $\pm$ 0.01 b  |
|                                                                   | QUSI | Drought |         |           |                      | 1.039 $\pm$ 0.00 b | 1.030 $\pm$ 0.00 a | 1.030 $\pm$ 0.00 a  | 1.261 $\pm$ 0.05 b  |
| REP ( $\lambda$ ) (nm)                                            | QULA | Control |         |           |                      | 715.7 $\pm$ 0.20 a | 715.6 $\pm$ 0.21 a | 716.1 $\pm$ 0.16 a  | 715.3 $\pm$ 0.20 a  |
|                                                                   | QUSI | Control |         |           |                      | 718.4 $\pm$ 0.19 b | 717.6 $\pm$ 0.52 b | 718.3 $\pm$ 0.26 a  | 717.8 $\pm$ 0.12 a  |
|                                                                   | QULA | Drought |         |           |                      | 716.1 $\pm$ 0.15 a | 716.2 $\pm$ 0.18 a | 716.3 $\pm$ 0.19 b  | 712.6 $\pm$ 0.58 b  |
|                                                                   | QUSI | Drought |         |           |                      | 717.1 $\pm$ 0.35 b | 717.2 $\pm$ 0.34 b | 715.2 $\pm$ 0.47 b  | 712.0 $\pm$ 0.93 b  |

$P < 0.01$ . Differences between  $\Psi_{pd}$  and  $\Psi_{md}$  were significant ( $P < 0.0001$ ) according to a GLM test. *Quercus sideroxyla* had significantly higher  $\Psi_{pd}$ ,  $\Psi_{md}$ , and RWC than *Q. laceyi* ( $P < 0.01$ ) at the onset of the experiment. Species differences in  $\Psi_{pd}$  and  $\Psi_{md}$  continued throughout the drought, with *Q. laceyi* having consistently lower  $\Psi_{pd}$  and  $\Psi_{md}$  than *Q. sideroxyla* ( $P < 0.05$ ). Treatment-level differences in  $\Psi_{pd}$  and  $\Psi_{md}$  were significant ( $P < 0.05$ ) beginning in wk 4 (Table 2).

**Photosynthesis, stomatal conductance, intracellular  $\text{CO}_2$ , and chlorophyll fluorescence**—All ANOVAs were significant at  $P < 0.0001$  (Table 1) for  $A_{max}$ ,  $g$ , and  $F_v/F_m$ . Intracellular  $\text{CO}_2$  (Ci) concentrations were not significantly

different between species, treatments, or sampling intervals, and they had an overall mean of 112.1 ( $\pm 7.95$  SE)  $\mu\text{mol CO}_2\cdot\text{mol air}^{-1}$ . Species differences in photosynthesis and stomatal conductivity were evident from the onset of the experiment ( $P < 0.001$ ), and  $A_{max}$  and  $g$  were higher for *Q. sideroxyla* than for *Q. laceyi* throughout the experiment (Table 2). Species-level differences in  $A_{max}$  and  $g$  declined in latter weeks of the drought, while treatment-level differences increased, with significant differences ( $P < 0.05$ ) occurring from the 4th wk on.  $A_{max}$  values were lower for *Q. laceyi* drought treatment seedlings during the first two sampling intervals due to random variability. This was probably the result of this species' existence on a wider range of sites relative to *Q. sideroxyla*. A nonsignificant decline in  $A_{max}$

TABLE 2. Continued.

| (C) Growth measure | Species | Treatment | Week 0 | Week 2         | Week 4         | Week 6 | Week 7         |
|--------------------|---------|-----------|--------|----------------|----------------|--------|----------------|
| No. leaves         | QULA    | Control   |        | 17.67 ± 0.87 a | 22.22 ± 0.94 a |        | 18.44 ± 1.03 a |
|                    | QUSI    | Control   |        | 20.30 ± 1.01 a | 23.50 ± 1.49 a |        | 21.30 ± 0.77 a |
|                    | QULA    | Drought   |        | 16.27 ± 0.98 a | 18.09 ± 1.22 b |        | 15.09 ± 0.66 a |
|                    | QUSI    | Drought   |        | 16.80 ± 0.62 a | 18.30 ± 1.17 b |        | 17.42 ± 1.40 a |
|                    | QULA    | Control   |        | 17.67 ± 0.94 a | 22.22 ± 0.94 a |        | 18.44 ± 1.03 a |
|                    | QUSI    | Control   |        | 20.30 ± 1.49 a | 23.50 ± 1.49 a |        | 21.30 ± 0.77 a |
| Height (cm)        | QULA    | Drought   |        | 16.27 ± 1.22 a | 18.09 ± 1.22 b |        | 15.09 ± 0.66 b |
|                    | QUSI    | Drought   |        | 16.80 ± 1.17 a | 18.30 ± 1.17 b |        | 17.42 ± 1.40 b |
|                    | QULA    | Control   |        |                | 4.95 ± 0.56 a  |        | 3.54 ± 1.95 b  |
|                    | QUSI    | Control   |        |                | 4.37 ± 0.02 a  |        | 3.16 ± 0.56 a  |
|                    | QULA    | Drought   |        |                | 3.42 ± 0.45 a  |        | 3.68 ± 0.46 a  |
|                    | QUSI    | Drought   |        |                | 2.88 ± 0.02 a  |        | 4.70 ± 0.23 b  |
| Root collar (cm)   | QULA    | Control   |        |                |                |        | 1.44 ± 0.19 a  |
|                    | QUSI    | Control   |        |                |                |        | 1.93 ± 0.19 a  |
|                    | QULA    | Drought   |        |                |                |        | 1.09 ± 0.08 b  |
|                    | QUSI    | Drought   |        |                |                |        | 1.06 ± 0.08 b  |
|                    | QULA    | Control   |        |                |                |        | 0.43 ± 0.08 a  |
|                    | QUSI    | Control   |        |                |                |        | 0.45 ± 0.04 a  |
| Root mass (g)      | QULA    | Drought   |        |                |                |        | 0.25 ± 0.03 b  |
|                    | QUSI    | Drought   |        |                |                |        | 0.29 ± 0.03 b  |
|                    | QULA    | Control   |        |                |                |        | 4.74 ± 0.57 a  |
|                    | QUSI    | Control   |        |                |                |        | 4.69 ± 0.50 a  |
|                    | QULA    | Drought   |        |                |                |        | 5.25 ± 0.56 a  |
|                    | QUSI    | Drought   |        |                |                |        | 4.36 ± 0.45 a  |

Note:  $A_{\max}$  = maximum photosynthesis;  $g$  = stomatal conductance;  $F_v/F_m$  = chlorophyll fluorescence to maximal chlorophyll fluorescence; chlNDI = chlorophyll normalized difference index; NDVI = normalized difference vegetation index; REP = red edge position; RWC = relative water content; SIPI = structure independent pigment index; WBI = water band index;  $\Psi_{pd}$  = pre-dawn water potential;  $\Psi_{md}$  = mid-day water potential.

occurred in control seedlings beginning on wk 4; the decline was probably the result of leaf aging during the experiment because all repeated photosynthesis measurements were taken on the same leaf. A positive relationship existed between  $A_{\max}$  and  $g$  for the pooled data set (Fig. 2), indicating an increase in stomatal conductance with increased photosynthesis. The ratios of  $F_v/F_m$  were much less sensitive than  $A_{\max}$  and  $\Psi$ , and they differed little between treatments or species until wk 6 and 7 of the drought (Table 2). Chlorophyll fluorescence in *Q. sideroxyla* declined first during wk 6 of the drought, while *Q. laceyi* had no significant decline in chlorophyll fluorescence until wk 7 ( $P < 0.05$ ).

**Spectral reflectance**—Reflectance spectra varied between species, although spectra for both species were characterized by a broad peak at 550 nm, a trough at 680 nm, a sharp increase through the red edge around 700 nm, followed by another broad peak at 760 nm, and a gently decreasing plateau above 750 nm (Fig. 3). Averaged across all control treatment seedlings and time intervals, *Q. laceyi* reflectance was higher than *Q. sideroxyla* for all wavelengths. Differences between control and drought treatments were observed 6 and 7 wk after drought onset (Fig. 3). The drought resulted in increased reflectance for both species relative to control treatments at all wavelengths and in a broad peak in reflectance that ranged across the visible light spectrum (400–700 nm).

Reflectance ratios were calculated to illustrate the differences in the reflectance spectra between species and treatments for wk 6 and 7 of the drought (Fig. 4). The reflectance ratios were calculated using the mean reflectance of each species–treatment combination, and they included drought *Q. laceyi*: control *Q. laceyi* (DL : CL); drought *Q. sideroxyla*: control *Q. sideroxyla* (DS : CS); drought *Q. sideroxyla*: drought *Q. laceyi* (DS : DL);

control *Q. sideroxyla*: control *Q. laceyi* (CS : CL). Major differences were similar for wk 6 and 7 of the drought. The two species' drought to control ratios were the highest (DS : CS and DL : CL) with peaks at 660 and 480 nm, and the change in reflectance due to drought was greater for *Q. sideroxyla* than for *Q. laceyi*. Species differences in reflectance in drought and control treatments were similar (DS : DL and CS : CL), with increases in reflectance near 680 nm.

**Reflectance indices**—The treatment effect was significant ( $P < 0.05$ ) for all reflectance indices (Table 1). Species effects

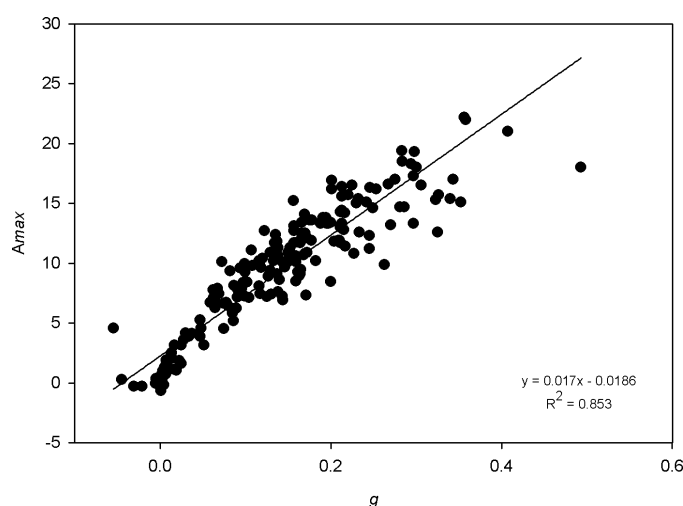


Fig. 2. Relationship between stomatal conductance ( $g$ ) and maximum photosynthesis ( $A_{\max}$ ) for pooled samples.

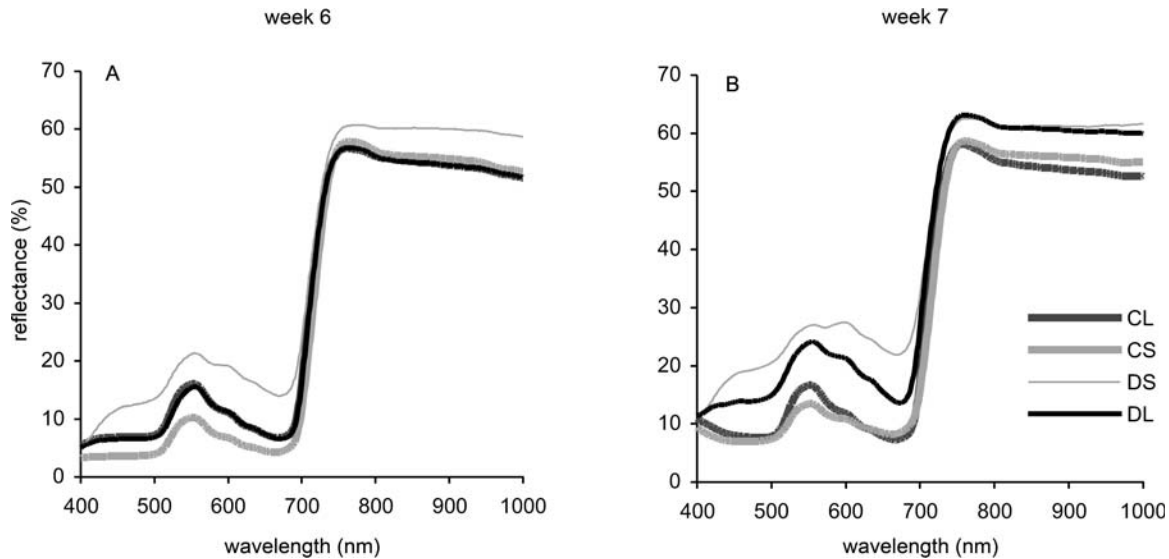


Fig. 3. Reflectance spectra of species-treatment combinations 6 wk (A) and 7 wk (B) after the onset of the drought experiment. Abbreviations: CL, control, *Quercus laceyi*; CS, control, *Q. sideroxyla*; DL, drought, *Q. laceyi*; DS, drought, *Q. sideroxyla*.

were significant ( $P < 0.05$ ) for SIPI,  $\text{chlNDI}$ , and NDVI but not for WBI. Time since watering was also significant for all indices except SIPI. Species, treatment, and time effects were evident in  $\text{chlNDI}$  values ( $P < 0.05$ ) (Table 2). Like the other measures of seedling performance, the reflectance indices identified significant differences between species at the beginning of the experiment, with *Q. sideroxyla* having significantly ( $P < 0.05$ ) higher values than *Q. laceyi* for all indices (Table 2). Species-level differences diminished over the course of the experiment and were replaced by treatment-level differences ( $P < 0.05$ ).

The red edge  $\lambda$  differed significantly ( $P < 0.01$ ) by species, treatment, and time (Tables 1 and 2). *Quercus sideroxyla* had a higher REP than *Q. laceyi* at all samplings until wk 6 of the drought, when treatment-level differences became significant ( $P < 0.05$ ). The shift in REP toward shorter wavelengths in

drought seedlings began in wk 6, although treatment effects were most pronounced in wk 7.

**Growth and biomass allocation**—Treatment effects on plant height were evident in wk 4 and 7 ( $P < 0.05$ ) (Tables 1 and 2). *Quercus sideroxyla* had significantly more leaves than *Q. laceyi* (Tables 1 and 2), but there were no significant treatment effects on leaf number. Control seedlings had significantly ( $P < 0.001$ ) thicker root collar diameters than drought seedlings (Tables 1 and 2).

Control seedlings had significantly greater ( $P < 0.01$ ) root and shoot biomass than drought seedlings, and control *Q. sideroxyla* had greater ( $P < 0.001$ ) dry root mass than *Q. laceyi* (Table 1). Decline in root and shoot mass was significantly greater ( $P < 0.01$ ) with *Q. sideroxyla* than *Q. laceyi* (Fig. 5A, B). *Quercus sideroxyla* roots were longer and more branched than those of *Q. laceyi*, and control seedling roots were longer

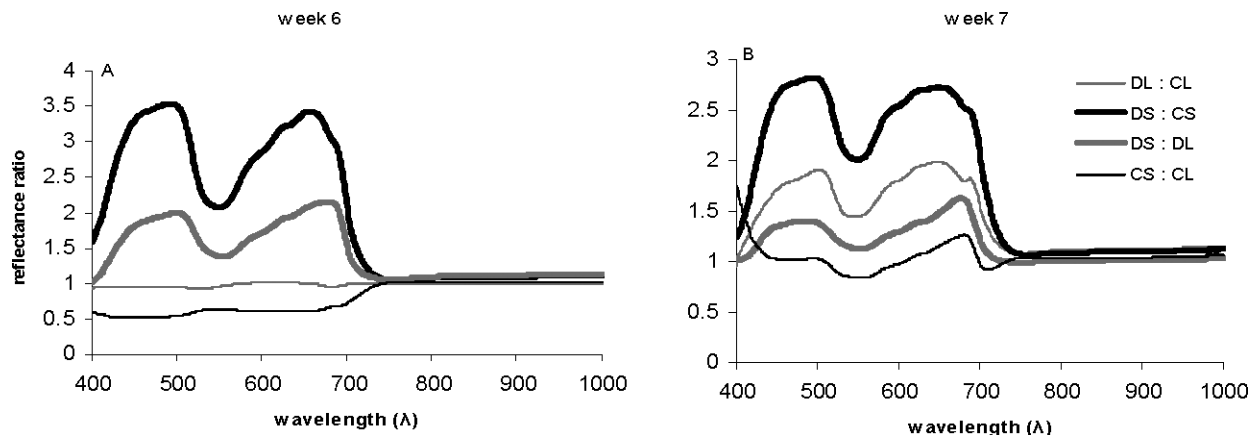


Fig. 4. Reflectance ratio spectra for species-treatment combinations 6 wk (A) and 7 wk (B) after the onset of the drought experiment. Abbreviations: CL, control, *Quercus laceyi*; CS, control, *Q. sideroxyla*; DL, drought, *Q. laceyi*; DS, drought, *Q. sideroxyla*.

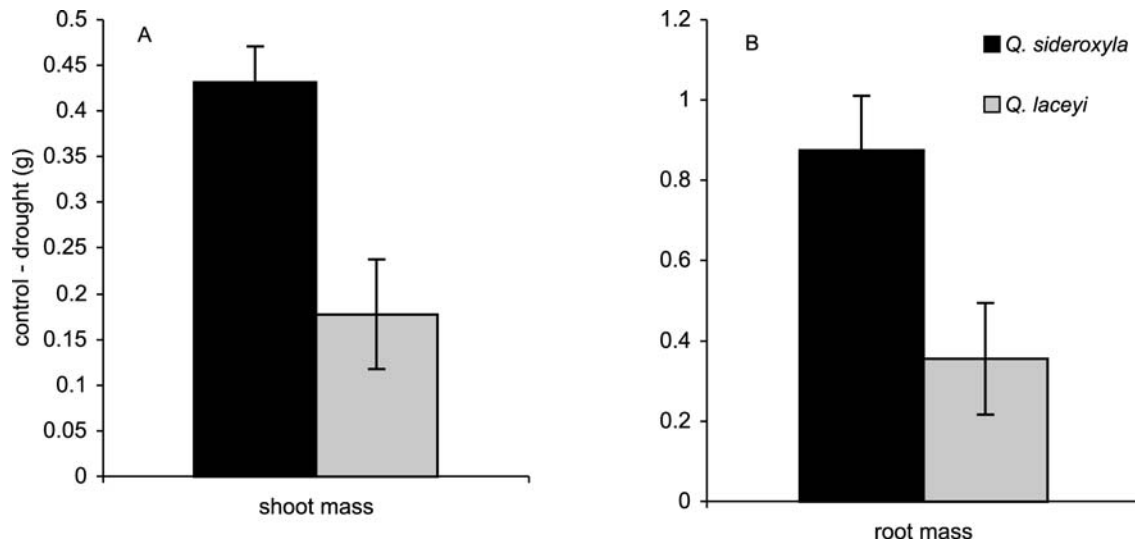


Fig. 5. Mean differences ( $\pm$ SE) between control and drought shoot (A) and root (B) biomass of seedlings of *Quercus laceyi* and *Q. sideroxyla* at the end of the 7-wk drought. Control and drought treatments differed significantly according to Tukey's comparisons ( $P < 0.01$ ).

and more branched than drought seedling roots. Root:shoot ratios did not differ significantly between species or treatments.

## DISCUSSION

Our results suggest that the oaks of northern Mexico have evolved to survive and reproduce under a range of climatic conditions. The species-level differences in oak physiology reflect each species' adaptation to local growing conditions, potentially explaining the differentiation and diversification of oaks within and between mountain ranges in Mexico. The similar drought responses of *Q. laceyi* and *Q. sideroxyla* suggest that both species are well adapted to drought, although their performance differs substantially under conditions that are not moisture-limited.

The dominance of *Q. sideroxyla* on cooler, wetter sites is explained by its higher photosynthesis, stomatal conductance, relative water content, and water potentials and its lower spectral reflectance relative to *Q. laceyi*, conferring competitive advantages on high-productivity sites at upper elevations. Likewise, the dominance of *Q. laceyi* on hotter, drier sites reflects its adaptation to the more extreme conditions typical at lower elevations. Our water potential ( $\Psi_{pd}$  and  $\Psi_{md}$ ) and reflectance ratio data corroborate these findings, by showing that *Q. laceyi* was better able to carry out key physiological processes than was *Q. sideroxyla* under drought conditions. This suggests that *Q. sideroxyla* avoids drought as a high-elevation site specialist, while *Q. laceyi* is a drought tolerant species that competes well under more extreme, low elevation conditions.

In general, higher water potential, higher photosynthetic rate, and higher relative water content equate to less drought tolerance in oaks (Nardini et al., 1999; Fotelli et al., 2000; Thomas and Gausling, 2000). While our results suggest that *Q. sideroxyla* is less drought adapted than is *Q. laceyi*, *Q. sideroxyla* remained highly resilient to drought, even with its initially higher water potentials, photosynthetic rate, and relative water content. A possible explanation for this pattern

is that the high variability in year-to-year precipitation in southwestern North America has caused species across a range of elevations to be adapted to wet and dry climatic cycles in order to persist long-term. Such findings further suggest that even less drought tolerant species such as *Q. sideroxyla* may be able to persist under future climate change scenarios (Houghton et al., 2001).

**Biomass allocation and growth**—The first and most sensitive response to water deficiency is a decrease in turgor and a slowing of the growth processes (Larcher, 2003). Plants of both species were shorter, had smaller root collars, and allocated less biomass to roots and shoots in the drought than in the control treatment because the rate of cell division and the duration of leaf expansion drop as cell turgor declines. Likewise, the drop in photosynthesis during the drought limited  $CO_2$  assimilation, thereby limiting growth.

**Water relations**—Plant water status is controlled by the relative rates of water absorption (uptake by roots) and water loss (transpiration), and a plant's drought tolerance depends upon its ability to tolerate internal water deficits. As soil moisture is depleted under drought, the leaf water content and the overnight recovery of  $\Psi$  are inhibited by progressive increases in liquid flow resistance by the soil or plant (Kozlowski and Pallardy, 1997). The lower RWC and water potentials ( $\Psi_{pd}$  and  $\Psi_{md}$ ) of *Q. laceyi* relative to *Q. sideroxyla* suggest that *Q. laceyi* is more drought tolerant than *Q. sideroxyla* because it is better able to endure extreme water deficits, although these differences in adaptation did not translate to species' differences in growth or biomass.

At wk 7 in the drought treatment,  $\Psi_{pd}$  and  $\Psi_{md}$  of both species were more negative (*Q. laceyi* =  $-6.6$  MPa and *Q. sideroxyla* =  $-4.5$  MPa) than water potentials of seedlings of oak species from other geographical regions including *Q. suber* ( $-3.0$  MPa) in Spain (Aranda et al., 2005) and Italy ( $-1.5$  MPa) (Nardini et al., 1999), *Q. robur* ( $-3.0$  MPa) and *Q. petraea* ( $-3.2$  MPa) in Germany (Thomas and Gausling, 2000), *Q. rubra* ( $-2.5$  MPa) in the northeastern United States (Kubiske

and Abrams, 1992; Cavendar-Bares, 2000), and several other oak species of the southwestern United States (*Q. buckleyi*, *Q. lobata*, *Q. macrocarpa*, *Q. muehlenbergii*, and *Q. shumardii*) (Balok and St. Hilaire, 2002).

Week 7  $\Psi_{pd}$  and  $\Psi_{md}$  compare more closely to other oaks, pinyon pines, and junipers from other arid regions of the Southwest, and oaks such as *Q. gambelii* and *Q. turbinella* appear to tolerate even lower  $\Psi_{md}$  (−7.3 MPa and −7.0 MPa, respectively) upon exposure to a similar 7-wk drought than did the seedlings in our study (Neilson and Wullstein, 1987). This suggests that oak trees from the southwestern United States and northern Mexico are more drought tolerant than oaks from other geographical regions, but that there is a lower limit of ~−7.0 MPa for oak water stress tolerance.

**Photosynthesis**—The decreases in  $\Psi_{pd}$  and  $\Psi_{md}$  in wk 6 and 7 of the drought induced stomatal closure, which decreased the supply of CO<sub>2</sub> to the mesophyll cells, and consequently, decreased the rate of leaf photosynthesis. Increases in internal water deficit are associated with disruption of biosynthetic activities, including the generation of the chlorophyll necessary for photosynthesis (Fitter and Hay, 2002), and photophosphorylation and photosynthetic carbon metabolism may be particularly sensitive to dehydration because of the toxic effects of Mg<sup>2+</sup> concentrations that accompany the removal of water from chloroplasts in dehydrating leaves (Kozłowski and Pallardy, 1997).

The higher photosynthetic rates of *Q. sideroxyla* relative to *Q. laceyi* also correspond to their elevational distributions. Adiabatic lapse rates and increases in relative humidity with increasing elevation can ameliorate climates in xeric environments (Barry, 1992; Robinson et al., 1999; Whiteman, 2000), providing more favorable growing conditions for plants at higher elevations. The pattern of increased photosynthesis with increasing elevation in our study is the reverse of the patterns observed in other alpine environments near upper treeline (Tranquillini, 1979). In the Southwest, the harshest sites are at low elevations, where lower treelines are limited by moisture and temperature (VanDevender, 1995). Lower temperatures and higher relative humidity at high elevations can therefore explain the pattern of high photosynthesis by *Q. sideroxyla* relative to *Q. laceyi*, and the lower maximal photosynthesis of *Q. laceyi* is likewise explained by its distribution in a more water-limited environment that demands lower maximum metabolic rates.

**Chlorophyll fluorescence**—The reduction in  $F_v/F_m$  during the drought reflects a decline in the efficiency of light use for electron transport by PSII. Under drought conditions, the decreases in photosynthesis are accompanied by a decrease in the rate of consumption of ATP and NADPH for CO<sub>2</sub> assimilation, which can reduce the rate of linear electron transport from the quinone acceptors of PSII, and consequently, in  $F_v/F_m$  (Baker and Rosenqvist, 2004). The decline in  $F_v/F_m$  of *Q. sideroxyla* in wk 6 of the drought and a similar decline by *Q. laceyi* in wk 7, support our findings from the water relations data that low elevation *Q. laceyi* is more drought tolerant than high elevation *Q. sideroxyla*. These data suggest that *Q. laceyi* can better compensate for decreases in the use of ATP and NADPH in photosynthesis metabolism resulting from drought.

**Spectral reflectance**—Leaf optical properties are well correlated with vegetation water content (Sims and Gamon, 2003) and leaf pigment content (Curran, 1989; Gamon and Surfus, 1999; Sims and Gamon, 2002), and reflectance is known to shift in response to environmental stress (Peñuelas et al., 1994; Rock et al., 1998; Carter and Knapp, 2001; Thorhaug et al., 2006). The higher reflectance spectrum of *Q. sideroxyla*, along with the higher chlNDI index and reflectance ratios of control seedlings, suggest that *Q. sideroxyla* has a higher concentration of plant pigments than *Q. laceyi*, which relates to its higher maximum photosynthetic capacity.

Changes in the reflectance properties of drought-stressed seedlings are the product of decreases in plant water balance and photosynthetic pigment concentrations. The drop in WBI in latter weeks of the drought indicated decreased plant RWC (Peñuelas et al., 1997; Sims and Gamon, 2003). Decreases in chlNDI, increases in SIPI, the shift of the REP to shorter wavelengths, and the increases in reflectance observed over a broader portion of the visible spectrum are typical plant responses to stress (Peñuelas et al., 1994; Carter and Knapp, 2001; Richardson et al., 2001; Richardson and Berlyn, 2002; Thorhaug et al. 2006), although the position of the red edge was high compared to other studies (i.e., Richardson et al., 2001; Richardson and Berlyn, 2002). Furthermore, the reflectance ratios between species in the drought and control reflectance spectra had noticeable peaks in the 680 nm region, suggesting a greater shutdown of PSII in *Q. sideroxyla* than in *Q. laceyi* during wk 6 and 7.

While photosynthetic rates tended to increase with oak elevational distribution, the reflectance spectra and reflectance ratios show an opposite trend, with lowland *Q. laceyi* having higher reflectance than upland *Q. sideroxyla*. This pattern is consistent with work in mountains of the northeastern United States (Richardson et al., 2001; Richardson and Berlyn, 2002), and the observed differences in spectral reflectance also probably relate to variation in the two species' growing conditions. The reflectance data suggest that the light green leaves of *Q. laceyi* have lower chlorophyll concentrations, which correspond both to its lower photosynthetic rate and its distribution on hot, dry, low-elevation sites. In contrast, the lower reflectance spectrum of *Q. sideroxyla* corresponds to higher chlorophyll absorption and higher photosynthetic capacity, most likely related to its distribution on cooler, wetter, high-elevation sites.

**Application of greenhouse results to field conditions**—The application of greenhouse experiments to field conditions warrants discussion (e.g., Harper, 1977), because the field environment can differ dramatically from controlled greenhouse conditions. We addressed this by mimicking natural patterns of oak germination, growth, and drought timing in the southwestern United States and northern Mexico.

Results from this study correspond to other field-based studies of plant water potential in the southwestern United States (i.e., Lajtha and Barnes, 1991; Lajtha and Getz, 1993; Linton et al., 1998), suggesting that our experiment approximated field soil moisture conditions. Seedling survival over the course of the acute drought was similar to other acute drought studies (Neilson and Wullstein, 1987; Lajtha and Barnes, 1991; Barton and Teeri, 1993; Lajtha and Getz, 1993; Linton et al., 1998; Balok and St. Hilaire, 2002). Although pots are known to constrain root elongation and subsequent water

uptake, roots of the seedlings in this study failed to reach the bottom of their pots during the experiment.

### Conclusions

Results from this study demonstrate the physiological mechanisms responsible for the observed elevational separation and niche partitioning of *Q. sideroxyla* and *Q. laceyi* in the Sierra del Carmen, Coahuila, Mexico. *Quercus laceyi* is a drought tolerant species that can grow under the high water stress conditions typically observed at low elevations during summer months in northern Mexico, while *Q. sideroxyla* is a drought avoider species that grows poorly under extreme water stress and is therefore restricted to higher elevations with lower temperatures and higher relative humidity. However, both species are adapted to dry conditions, suggesting that they can survive under a range of climate scenarios. The differentiation of these two oaks according to elevation suggests that similar mechanisms may be responsible for the diversification of the genus *Quercus* across Mexico and the rest of North America. Future studies that investigate the niche partitioning of oaks across other mountain ranges in Mexico are warranted to explain the high diversity of this genus across Mexico.

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